Continental J. Pharmaceutical Sciences 5: 1 - 10, 2011 ISSN: 2141 - 4149

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PRELIMINARY INVESTIGATION OF A HERBAL SOAP INCORPORATING *Cassia senna*(L) Roxb Leaves and *Ageratum conyzoides* Linn WHOLE PLANT POWDERS.

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ABSTRACT
In traditional medicine practice the locally made soap is a vehicle for drug application and drugs are usually incorporated in powder form. Many herbal soaps being made incorporate the extract of the medicinal plant, but this makes the soap very expensive and not easily produced for the use of the majority of people. This study investigates the activity of a herbal soap incorporating two medicinal plants , *Senna alata* (L) Roxb (Ceasalpinaceae) and *Ageratum conyzoides* Linn(Asteraceae) which are well known for their use in the treatment of skin diseases and their antimicrobial activity, comparing it to the extracts of the plants. The study also determines the optimum concentration of the herbs to be incorporated into the soap. Three soaps containing 3,5 and 8 % of the moderately fine powder of the two medicinal plants were prepared using the normal cold method for preparing hand and body soap with NaOH and Palm kernel oil. Extract of the two plants was prepared by cold extraction with methanol and concentrated *in vacuo*. The soaps and the extract were tested for antimicrobial activity against typed organisms and clinical isolates, gram negative, gram positive organisms and fungi. The results showed that the soap at 5% herbal content was optimal. The soaps showed activity against mainly the gram positive organisms and fungi. The activity of the extract was not different from that of the soaps and therefore shows no advantage in using the extracts to prepare the herbal soap.

KEYWORDS , herbal soap, *Senna alata*, *Ageratum conyzoides*, antimicrobial

INTRODUCTION

In traditional medicine soaps are a very common vehicle for application of medicinal plants especially for external use and also for the treatment of skin diseases (Ajaiyeoba *et al*, 2003; Ahmed *et al*, 2005;Ajose, 2007). Locally manufactured soap is traditionally used. Locally manufactured soap is made from lye obtained from ash of burnt cocoa husks, plantain peels, palm wastes, wood and other plant debris and is known to have some antimicrobial properties (Lamikanra and Allwood, 1977;Adebiyi 1980; Moody *et al*.2004).

These soaps are used in formulation of various traditional herbal medicaments intended for topical use. The soaps perform the function of an ointment base and other components or the active ingredients are added in powder form. The soaps are used principally for their alleged antiseptic properties and such are used to bath the affected areas before application of herbal preparations(Ajose, 2007)

The incorporation of plant materials and extracts with activity against various skin conditions from eczema to purities and other more serious dermatological ailments, into soap is one of the major ways medicinal plants are utilized (Kareru *et al* 2010). The focus of this study was to develop a herbal soap using the basic soap ingredients, incorporating herbs which are easily available in the environment, in powder form and assess the antimicrobial activity of the soap and extracts of the plants. Plant extracts and powder have been incorporated into soaps and were found active Esmone *et al*,2008; Oladele *et al*, 2010).

The plants chosen for the formulation of the herbal soap are two well known plants which have been investigated for various activities, especially for their antimicrobial activity. *Senna alata* (L)Roxb leaf(Caesalpinaceae) is naturally abundant in Bayelsa state, Nigeria. *Agreratum conyzoides* Linn whole plant (Asteraceae)is found almost throughout the year in Bayelsa State, Nigeria as the rainy extends from March to November.

These two plants have been well researched by many workers and therefore qualified to be used in formulations of herbal drugs. There are pharmacopoeia standards set for *Senna alata* leaves in the Nigerian Herbal Pharmcopoeia (NHP,2008) *Senna alata* is also in the French Pharmacopeia (Hennebelle *et al*,2009)

Ageratum conyzoides is a plant on the second list of plants to be included in the second volume of the Nigerian Herbal pharmacopoeia(NHP,2008).

Senna alata commonly called ringworm, 'craw craw' plant or candle stick plant used for dermatitis, eczema ringworm gonorrhea (Dalziel, 1937, Irvine, 1961) is found in the rain forest and savannah areas in both the southern and northern parts of Nigeria. It is common in villages, wastelands and clearings and chiefly in the forest regions of Nigeria (Adjanohoun, *et al*1991

Senna alata leaf contains reduced anthraquinone; aloe-emodin, rhein glycoside and aloe-emodin glycoside (Rai,1978). Kaempferol 3- O-gentiobioside and aloe emodin were also obtained from the leaf(Hazni *et al* 2008). Twelve compounds were isolated from C. alata, which were identified as chrysoeriol, kaempferol, quercetin, 5,7,4'-trihydroflavanone , kaempferol-3-O-beta-D-glucopyranoside , kaempferol-3-O-beta-D-glucopyranosyl-(1-->6)-beta-D-glucopyranoside, 17-hydrotetratriacontane , n-dotriacontanol , n-triacontanol , palmitic acid ceryl ester, stearic acid , palmitic acid (Liu *et al*, 2009). Assay of leaves for flavonoid content by Akinmoladun *et al* (2010) showed the leaves contained 275.16=/-1.62 microg/ml quercetin equivalent and significant antioxidant activity. Cassiaindoline, an indole alkaloid was isolated from *Senna alata* and showed anti-inflammatory activity (Villaseñor *et al* 2009). Methanolic extract of the leaves of *Senna alata* exhibited inhibition against Methicillin Resistant *Staphylococcus aureus*. Chrysophanol, physcion and kaempferol have also been isolated from the root of *Senna alata* (Fernand *et al*, 2008).

Senna alata leaves have shown antimicrobial and laxative activities (Elujoba *et al* 1989). The leaf and flower of *S. alata* showed antimicrobial activity on unspecified Gram-positive bacteria and against *Trichophyton rubrum* and *Basidiobolus haptosporus*. Oil extracted from the leaf had inhibitory effects on Gram-positive and Gram-negative bacteria including *Pseudomonas* sp., *Staphylococcus aureus* and *Escherichia coli* (Ogunti *et al* 1991).Leaf extracts against were active against *Propionibacterium acnes,* and *Staphylococcus epidermidis* which are the organisms implicated in acne (Chomnawang *et al,* 2005).Other investigations showed leaf extract to be active against *Trichophyton tonsurans Microsporum soudanense* and more active than griseovulvin and clotrimazole (Eja *et al*, 2009). Leaves have been used to treat dermatophilosis infected animals and there was no recurrence (Ali- Emmanuel *et al* 2003).

A. conyzoides is widely utilized in traditional medicine by various cultures worldwide, although applications vary by region. It is used to treat pneumonia,wounds and burns (Durodola,1977). It has also been used as a bacteriocide, antidysenteric, and antilithic (Borthakur and Baruah. 1987). It is used to treat fever, rheumatism, headache, and colic (Menut,1993; Vera, 1993) . *A. conyzoides* has quick and effective action in burn wounds and is recommended by Brazilian Drugs Central as an antirheumatic (Brasil, Ministério da Saúde, Central de Medicamentos, 1989; Akinyemi *et al* 2005 ; Lans, 2007)

Flavonoids, alkaloids, coumarins, tannins and essential oils have been obtained from *A conyzoides*. (Jaccoud,1961; Oliveira *et al* 1993.) Compounds which have been isolated from *A conyzoides* are conyzorigum, a cromene (Borthakur and Baruah. 1987), precocene I and precocene II, which have been shown to affect insect development, as antijuvenile hormones, resulting in sterile adults (Ekundayo *et al*, 1988).Other compounds, flavones, ageconyflavones A,B,C, hexamethoxy flavone, cumarinic compounds including 1-2 benzopirone and alkaloids of the pirrolizidinic group have been isolated(Trigo *et al*, 1988). 51 terpenoid compounds were identified from volatile oil from *A.conyzoides*, including precocene I and precocene II (Gonzales, 1991.)11 cromenes in the essential oils, including 6-angeloyloky-7-methoxy-2,2-dimethylcromene were identified (Vera, 1993). Ageratocromene, and beta cariophylene were also obtained from *A conyzoides* (Horie *et al*, 1993). Ether and chloroform extracts of the plant had inhibitory activity against *Bacillus subtilis*, *Staphylococus aureus*, *Escheriachia coli* and *Pseudomonas aeruginosa* (Almagboul *et al* 1985). Aqueous extracts showed analgesic action and whole plant extracts had muscle relaxing activities (Achola *et al*, 1994).

MATERIALS AND METHODS

Plant Collection

Senna alata leaves were collected from the Department of Pharmacognosy and Herbal Medicine, Niger Delta University, Nigeria, medicinal plant garden. The leaves were air dried for a few days and then ground. The powder stored in well sealed containers away from light for further use.

Ageratum conyzoides was collected from the grounds of the College of Health Sciences, Niger Delta University, Nigeria. The soil was removed from the roots and washed with distilled water. The whole herb was air dried for a few days and ground and stored in well sealed containers and stored away from light for further use.

Plant Powder

The Plant material to be introduced was sieved to give a moderately fine powder according to the definition in the Quality control methods for medicinal plant materials by WHO. All particles will pass through a No. 355 sieve(Nominal aperture size 0.355mm) and not more than 40% through a No. 108 sieve Nominal aperture size 0.180mm). After obtaining the grade of powder desired, the powdered plant materials were weighed in equal amounts. The two plant materials were then sifted together to mix completely. The amount to be put into the soap mixture was then weighed out.

Plant Extraction

One hundred grams each of the two plant materials were weighed and extracted successively for 72 hours in the cold with methanol. The extract was filtered and the extract concentrated *in vacuo* at room temperature using a rotary evaporator. The extract was stored at 4^{o}C till needed.

Soap Materials

Palm kernel oil (Pko)was obtained from local producers in the Osun State, Nigeria as the one obtained, locally in Baylesa State Yenagoa, Nigeria was full of burnt debris.
Water – distilled water
NaOH- BDH analytical quality

Methodology

The following soap formula was used to make a normal hand and body soap. This was scaled down to produce laboratory size.

2.3 liters of Pko
0.36kg of NaOH
0.9 liters of Water

The cold method of soap making was used to prepare the herbal soap. The NaOH was dissolved in the water in a beaker and allowed to cool, poured gradually into the oil with continuous stirring. The plants materials were added gradually with stirring with a glass rod and the mixture was then poured into a rectangular pan and left to set.

Soap containing three different percentages of plant material 3, 5 and 8%w/w were produced. The soaps were cut into squares of 5cm by 5cm for ease of handling.

Antimicrobial assessment

The three formulations of soap and the extracts of the two plants were subjected to anti microbial tests using gram negative and gram positive organisms and fungi. Typed and pathogenic organisms were used.

METHODOLOGY

The agar-dilution method for the determination of minimum inhibitory concentration of antimicrobial agents approved by CLSI was employed (CLSI 2008). 5g of the herbal soap was suspended and dispersed in 10ml of sterile distilled water to obtain a stock suspension containing 500mg/ml. From this stock, dilutions were made by adding 1ml of the suspension to 9ml of sterile molten Mueller Hinton agar in Petri-dishes to obtain a plate containing the soap at a concentration of

50mg/ml (50g/l) and allowed to set and harden. Similar dilutions were carried out to obtain plates containing the other different concentrations of the soap tested. Plates were prepared in duplicates and plates prepared without including the soap were used for negative control tests. The set plates were dried in an oven at 60^{o}C for 15 minutes before inoculating with overnight broth culture of each bacterial test organisms using a multi-inoculator. Similar plates were prepared using Sarbouraud dextrose agar (SDA) for testing the fungi. Inoculated plates were incubated at 37^{o}C for bacteria and 25^{o}C for fungi for 72 hours. Plates were examined for growth to determine presence or absence of inhibition.

RESULTS AND DISCUSSION

Plant extraction

The yield of extract was 9.8%w/w.

Antimicrobial Assay

The formulation is active against Sa and Sc at 8%, 5%, and 3% but more potent at 5% (Table 1), this indicates that there is possible synergic action at this concentration, while at higher concentration the activity is low, this may be due to antagonistic activity.

The formulation is more active against Gram positive bacterial (both typed and Clinical strains) and Fungi at the concentration tested (Tables 2,3and 4). No activity was shown against Gram negative organisms. This is expected as gram negative bacteria , particularly *Ps. aeruginosa* is known to be resistance to various antimicrobial agents(Quinn *et al*.1986). For *B. subtilis* the soap gave a different picture by being more active at 5% than 8% (Tables 3 and 4).

Investigation by Esimone *et al*(2008) shows the antimicrobial effect of the soap formulated with ethanolic extract of Senna alata exhibited excellent antimicrobial activity, activity was predominantly against Gram-positive organisams and ooprtunistic yeast. Oladele *et al* (2010)incorporated the *Senna alata* leaf powder into the soap and used it to treat skin infection by *Tinea versicolor* and *Tinea corporis* It is clear from their study that the plant *Senna alata* is active against organisms that cause skin diseases. This study is incorporating the plant material itself and comparing this to the activity of the extract. The incorporation of plant material into soap is not new as this is normal procedure in traditional medicine. Plant materials are incorporated in the traditional soap known as 'Black soap'.

The study has shown a good method of determining the concentration of herbs which may be needed for the formulation of a herbal antimicrobial soap. In this case the optimum concentration could be determined. The soap need not contain more than 5%W/W of the herbs.

There is no doubt that the soaps were active and showed antimicrobial activity against gram positive bacteria and fungi. This is a good development as it means that in formulating herbal soaps it is possible to incorporate the plant material and still get the desired results. This has an important implication to the cost of producing herbal soaps which are known to be expensive due to having to extract the plant materials using expensive solvents. This will no doubt reduce the cost of production of the herbal soap, making it more assessable to majority of the populace. The rational for investigating this method of the use of herbs in soap is justified. Two medicinal plants well known for their activities are incorporated, this gives a broader spectrum of activity. Also, it is seen that there could be possibilities of synergism which is good, but also antagonism could occur between compounds, which may cause problems of drug-drug interactions.

CONCLUSION

From the antimicrobial assessment of the extract of the plants and the soaps the optimum percentage of the plant materials to be used for the herbal soap was determined. Further investigations of the clinical use of the herbal soap with the incorporated plant materials in its crude form are still undergoing clinical trials.

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Received for Publication: 05/02/2011
Accepted for Publication: 14/03/2011

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Table 1 Results of antimicrobial Assay of Crude Extracts and Herbal soap at 3,5,and 8% Herbal Content

	Concentration(g/L)						
		025	0.50	1.00	10.00	20.00	40.00
A Crude Leaf Extract	Ps	+	+	+	+	+	-
	Ec	+	+	+	+	+	-
	Sa	+	+	+	-	-	-
	Sc	+	+	+	-	-	-
	Bs	+	+	+	+	+	+
	Bc	+	-	-	-	-	-
B (8%)	Ps	+	+	+	+	+	+
	Ec	+	+	+	+	+	-
	Sa	+	+	-	-	-	-
	Sc	+	+	-	-	-	-
	Bs	+	+	+	+	+	-
	Bc	-	-	-	-	-	-
C (5%)	Ps	+	+	+	+	+	-
	Ec	+	+	+	+	+	-
	Sa	-	-	-	-	-	-
	Sc	+	-	-	-	-	-
	Bs	+	+	+	+	+	-
	Bc	-	-	-	-	-	-
D(3%)	Ps	+	+	+	+	+	-
	Ec	+	+	+	+	+	-
	Sa	+	-	-	-	-	-
	Sc	+	+	-	-	-	-
	Bs	+	+	+	+	-	-
	Bc	-	-	-	-	-	-

Key:Ps: *Pseudomonas aeruginosa* (ATCC 19429), Ec: *Escherichia coli* (NCTC 10416), Sa: *Staphylococcus aureus* (NCTC 6571), Sc: *Staphylococcus aureus* (Clinical isolates), Bs: *Bacillus subtilis* (NCTC 8326), Bc: *Bacillus cereus* (Clinical isolates).+: Growth observed, -: Inhibition

Table 2 Results of Antimicrobial Assay of Herbal soap against clinical isolates at 8% Herbal content

8%										
Conc g/L	B (NCTC8326)	E (NCTC10416)	Ps (ATCC19429)	S (NCTC6571)	S(HR1)	S(04TW)	S2	S3	S(ATCC)	Cp(NCYC6)
50	+	+	+	-	-	-	-	-	-	-
40	+	+	+	-	-	-	-	-	-	-
30	+	+	+	-	-	-	-	-	-	-
20	+	+	+	-	-	-	-	-	-	-
10	+	+	+	-	-	-	+	-	-	-
5	+	+	+	-	-	-	+	+	-	-
1	+	+	+	-	-	-	+	+	-	+
0.5	+	+	+	+	+	-	+	+	-	+
0.25	+	+	+	+	+	-	+	+	+	+

KEY: CLINICAL ISOLATES, B: *B. subtilis*, E: *E. Coli*, Ps: *Pseudomonas aeruginosa*, S(NCTC): *Staphylococcus aureus*, S(ATCC): *Staphylococcus aureus*, Cp: *Candida pseudotropicalis*. +: no inhibition (Growth observed), -: inhibited (MIC).

Table 3 Results of Antimicrobial Assay of Herbal soap against clinical isolates at 5% Herbal content

5%										
Conc g/L	B (NCTC8326)	E (NCTC10416)	Ps (ATCC19429)	S (NCTC6571)	S(HR1)	S(04TW)	S2	S3	S(ATCC)	Cp(NCYC6)
50	-	+	+	-	-	-	-	-	-	-
40	-	+	+	-	-	-	-	-	-	-
30	-	+	+	-	-	-	-	-	-	-
20	-	+	+	-	-	-	-	-	-	-
10	-	+	+	-	-	-	+	-	-	-
5	+	+	+	-	-	-	+	+	-	-
1	+	+	+	+	+	+	+	+	-	+
0.5	+	+	+	+	+	+	+	+	+	+
0.25	+	+	+	+	+	+	+	+	+	+

KEY: CLINICAL ISOLATES, B: *B. Subtilis*, E: *E. Coli*, Ps: *Pseudomonas aeruginosa*, S(NCTC): *Staphylococcus aureus*, S(ATCC): *Staphylococcus aureus*, Cp: *Candida pseudotropicalis*. +: no inhibition (Growth observed), -: inhibited (MIC).

Table 4: Results of Antimicrobial Assay of Herbal soap against clinical isolates at 3% Herbal content

3%										
Conc g/L	B (NCTC8326)	E (NCTC10416)	Ps (ATCC19429)	S (NCTC6571)	S(HR1)	S(04TW)	S2	S3	S(ATCC)	Cp(NCYC6)
50	+	+	+	-	-	-	-	-	-	-
40	+	+	+	-	-	-	-	+	-	-
30	+	+	+	-	-	-	-	+	-	-
20	+	+	+	-	-	-	-	+	-	-
10	+	+	+	-	-	-	-	+	-	-
5	+	+	+	-	-	-	+	+	-	-
1	+	+	+	+	+	+	+	+	+	+
0.5	+	+	+	+	+	+	+	+	+	+
0.25	+	+	+	+	+	+	+	+	+	+

KEY: CLINICAL ISOLATES, B: *B. Subtilis*, E: *E. Coli*, Ps: *Pseudomonas aeruginosa*, S(NCTC): *Staphylococcus aureus*, S(ATCC): *Staphylococcus aureus*, Cp: *Candida pseudotropicalis*. +: no inhibition (Growth observed), -: inhibited (MIC).